Probiotic Biotherapy for Eradication of a Potential Pathogen in a Commercial Rat Breeding Facility

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Summary
This study defined a model for biotherapeutic eradication of β-haemolytic streptococci, group G, in a rat breeding unit (3100 rats) by use of a strain of *Lactobacillus reuteri*. The microbe was added to the rats' drinking water and the genitals of all rats were swabbed three times with a solution of the microbe. After this procedure undesirable streptococci were not recognized in any animal of this breeding farm.

Introduction
Some time ago, traces of β-haemolytic streptococci, group G were detected in vaginal samples from a breeding rats unit. As these microbes were on the "black list" of FELASA *(Federation of European Laboratory Animal Science Association)* recommendations (*Nicklas et al., 2002*), efforts had to be taken to eliminate them from the animals.
When we were contacted, three alternatives were taken into consideration:

i) exterminating all rats and establishment of a new breeding colony
ii) treating the rats with antibiotics or
iii) utilizing a probiotic bacterial strain as a "biotherapeutic" agent.

The third alternative was considered to be less expensive and less drastic (as compared to the two first ones) and was therefore taken into account. As we could not find any description in the literature how to proceed, we had to make our own protocol. We decided that

i) all animals should be exposed three times to the biotherapeutic agent,
ii) a probiotic strain isolated from rats should be used, and
iii) microbial controls should be performed after each treatment period.

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Materials and Methods
A total number of three thousand female rats and one hundred male rats of the Spraque Dawley strain had to be treated.
A stock culture of *Lactobacillus reuteri*, originally isolated from rats, was cultivated in MRS-broth at 37°C to yield a final concentration of approximately 10^9 CFU/ml. This culture was brought to the animal house where sterile cotton swabs were dipped in the cultures and then the vagina of all females and the penis of all males were swabbed, i.e. one new swab per animal. Simultaneously, *L. reuteri* was added to the rats' drinking water yielding a final concentration of 10^7 CFU/ml. These procedures were repeated three times, with a four weeks interval.
After each treatment, swab tests from 20 animals were investigated in a blind fashion for presence of group G streptococci.

Results
All animals remained superficially healthy throughout the observation period. After the first treatment period, group G streptococci were found on one of the samples investigated whereas all samples were free of group G streptococci after the second and the third treatments. In the year that followed, results from all general health checks of

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this animal farm demonstrated absence of group G streptococci.

Discussion

In clinical veterinary practice, the so-called "stamp-out" method, i.e. eradication of the whole exposed cohort of animals is still commonly used when some very virulent and strongly undesirable agents are found in any stock. A recent example was the presence of the bird flu virus in turkey farms in Great Britain. However, this method is seldom used when less virulent organisms, as most of those on the "black list" from the FELASA recommendations, are found in some few individuals of laboratory animals. In the present case, eradication of the whole cohort of Sprague Dawley rats would have given great inconvenience for many research groups all over Scandinavia.

In most European countries, the use of antibiotics when raising animals is strongly regulated. In the present case, the animals were not sick, but some few of them were harbouring an undesirable bacteria strain. Giving antibiotics to all rats for a substantial period of time might have led to success but at the expense of considerable disturbances in the normal flora and their interaction with the animals *(Hart et al., 2002)*, as well as to the possible development of antibiotic resistance. Additionally, we were not aware of any report describing eradication of group G streptococci from a large number of laboratory animals.

The concept of prophylactic use of probiotics for keeping individuals – or stocks – healthy, is a widely used approach in veterinary *(Collinder et al., 2003; Collado et al., 2007)* as well as human medicine *(Kallomäki et al., 2001; Gionchetti et al., 2006; Imase et al., 2007)*. A variety of microbial species are utilized world wide, such as lactobacilli, bifidobacteria, bacillus, clostridia, *Escherichia coli*, yeast and so on. It is often claimed that the probiotic to be used should originate from the normal flora of the type of animals to be treated *(Casas & Dobrogosz, 2000)*, and therefore, we decided to use a rat strain of *L. reuteri*. However, our success should not be taken as a strengthening of this claim since we have no explanation for the mechanisms of action behind the eradication of the streptococci. It might very well be that utilization of any lactobacilli could have given the same results**.**

The main message in this note is that use of probiotics, as biotherapeutic agents should be considered when undesirable bacteria are found to be present in the normal flora of laboratory animals.

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